A REPRESENTATIONAL APPROACH TO DNA ANALYSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 09/261,079 filed March 2, 1999, which is a continuation of application Ser. No. 09/115,061 filed July 14, 1998, now U.S. Pat. No. 6,159,713, which is a continuation of application Ser. No. 08/478,242 filed June 7, 1995, now U.S. Pat. No. 5,876,929, which is a continuation of application Ser. No. 08/149,199 filed November 9, 1993, now U.S. Pat. No. 5,501,964, which is a continuation-in-part of application Ser. No. 07/974,447 filed November 12, 1992, now U.S. Pat. No. 5,436,142.

GOVERNMENT LICENSE RIGHTS

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of contract OIG-5R CA39829-08 awarded by the National Institutes of Health.

INTRODUCTION

Technical Field

The field of this invention is DNA analysis.

Background

Comparative genomic DNA analysis holds promise for the discovery of sequences which may provide for information concerning polymorphisms, infectious DNA based agents, lesions associated with disease, such as cancer, inherited dominant and recessive traits, and the like. By being able to detect particular DNA sequences which have a function or affect a function of cells, one can monitor pedigrees, so that in breeding animals one can follow the inheritance of particular sequences associated with desirable traits. In humans, there is substantial interest in forensic medicine, diagnostics and genotyping, and determining relationships between various individuals. There is, therefore, substantial interest in providing techniques which allow for the detection of common sequences between sources and sequences which differ between sources.

The mammalian genome is extraordinarily large, having about 6×10^9 bp. The human genome project has initiated an effort to map and sequence the entire

PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Wigler *et al.*	Examiner:	Not assigned
Serial No.:	Not Assigned	Art Unit:	Not assigned
Filed:			
For:	**A REPRESENTATIONAL APPROACH TO DNA ANALYSIS**	**VERSION WITH MARKINGS TO SHOW CHANGES MADE**	

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

These marked-up versions of the claims and specification accompany the attached amendment under 37 CFR 1.312 for the above identified patent application.

REMARKS

In The Specification

On page 1, line 3, delete "This application is a continuation-in-part of application serial no. 07/974,447, filed November 12, 1993.", and insert the following text:

--This application is a continuation of application Ser. No. 09/261,079 filed March 2, 1999, which is a continuation of application Ser. No. 09/115,061 filed July 14, 1998, now U.S. Pat. No. 6,159,713, which is a continuation of application Ser. No. 08/478,242 filed June 7, 1995, now U.S. Pat. No. 5,876,929, which is a continuation of application Ser. No. 08/149,199 filed November 9, 1993, now U.S. Pat. No. 5,501,964, which is a continuation-in-part of application Ser. No. 07/974,447 filed November 12, 1992, now U.S. Pat. No. 5,436,142.--.

CERTIFICATE OF EXPRESS MAILING

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I hereby certify under 37 C F R. 1 10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" with sufficient postage on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D C 20231

(Signature)

Jeffrey M Libby

(Printed Name)

At page 1, line 5, insert the following text:

--GOVERNMENT LICENSE RIGHTS

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A substitute Page 1 is attached.

In The Claims

Cancel Claims 1-18.

Add the following new claims:

--19. (New) A DNA probe from a first eukaryotic source of DNA comprising at least one sequence difference from a related second eukaryotic source of DNA, said probe produced according to a method comprising the steps of:

substantially completely digesting separately the DNA from said first and said second source of DNA with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA, and said first source of DNA comprises tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said driver DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the

resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second round or successive round, whereby said DNA probe from said first eukaryotic source of DNA is produced.

20. (New) The DNA probe according to claim 19, wherein said first source and said second source of DNA are from the same individual.

21. (New) The DNA probe according to claim 19, wherein one of said first source and said second source of DNA comprises pathogen DNA.

22. (New) The DNA probe according to claim 21, wherein said pathogen DNA is viral DNA.

23. (New) The DNA probe according to claim 19, wherein said DNA is genomic DNA.

24. (New) The DNA probe according to claim 19, wherein said DNA is cDNA.

25. (New) The DNA probe according to claim 19, wherein said DNA probe is cloned.

26. (New) A kit comprising: a DNA probe according to claim 19.

27. A method of identifying sites that differ between two different related eukaryotic DNA sources, said method comprising the steps of:

i) hybridizing a probe obtained according to claim 19 to DNA from a first source and DNA from a second source

ii) determining the sequences to which said probe hybridizes, when said probe hybridized to the DNA from one but not both DNA sources.

28. A composition comprising a pooled plurality of DNA probes, each probe obtained from a first eukaryotic source of DNA and comprising at least one sequence difference from a related second eukaryotic source of DNA, said probe produced according to a method comprising the steps of:

completely digesting separately the DNA from said first and said second source of DNA with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA, and said first source of DNA comprises tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said driver DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second round or successive round.

29. (New) A method for detecting at least one genomic alteration occurring in a cancer cell as compared to a normal cell, said method comprising the steps of:

substantially completely digesting separately DNA from a first source and a second source each with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA obtained from a normal cell, and said first source of DNA comprises tester DNA obtained from a cancer cell,

wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said driver DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second round or successive round, whereby said genomic alteration occurring in a cancer cell is detected.

30. (New) The method according to Claim 31, wherein said cancer cell and said normal cell are obtained from the same individual.

31. (New) A method of identifying a pathogen DNA sequence incorporated into the genome of a eukaryotic cell, said method comprising the steps of:

substantially completely digesting separately the DNA from a first source and a second source of DNA, each with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA obtained from an uninfected eukaryotic cell, and said first source of DNA comprises tester DNA obtained from an infected eukaryotic cell, wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide

amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second round or successive round, whereby said probe specific for a pathogen DNA sequence is isolated.--

CONCLUSION

Should the Examiner have any questions regarding the above, the Examiner is invited to call the Undersigned.

Respectfully submitted,

Dated: August 22, 2001

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